Claims

1 A method of selecting one or more 1,2,4-benzotriazine-1,4-dioxides capable of in vivo hypoxia selective cytotoxicity, wherein said 1,2,4-benzotriazine-1,4-dioxide is selected if it is determined to have each of the following characteristics

(a) a solubility greater than or about 2mM in culture medium; and

(b) an HT29 anoxic $IC_{50}$ for a 4hr exposure to the 1,2,4-benzotriazine-1,4-dioxide of less than or about 40 μM; and

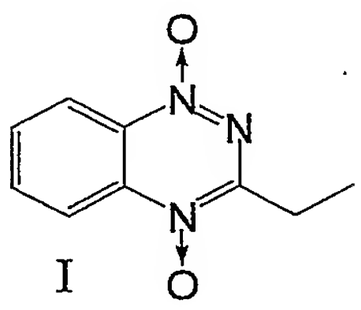
(c) a hypoxic cytotoxicity ratio (HCR) greater than about 20 for the HT29 cell line; and

(d) a penetration half distance (PHD) greater than or about 27 μm, and

(e) the area under the plasma concentration time curve for free 1,2,4-benzotriazine-1,4-dioxide (unbound to plasma proteins), $AUC_f$, is greater than about 2 times the HT29 anoxic $IC_{50}\times t$ where $IC_{50}\times t$ is the product of concentration × exposure time for 50% inhibition of cell proliferation

and wherein for said 1,2,4-benzotriazine-1,4-dioxide at least one of the characteristics (a) to (e) exceeds the activity of the equivalent characteristic of Tirapazamine.

2 A 1,2,4-benzotriazine-1,4-dioxide having in vivo activity and selected by the method defined in claim 1, with the proviso that Tirapazamine and compounds of Formula I and J



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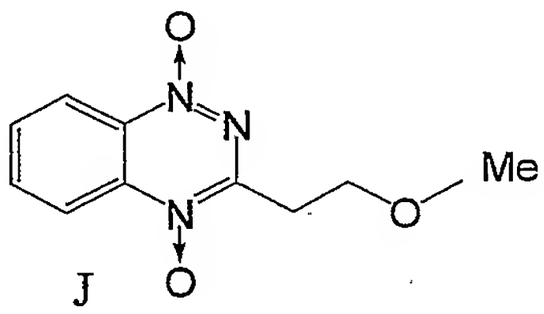


J

are excluded.

3 A 1,2,4-benzotriazine-1,4-dioxide compound as claimed in claim 2 selected from

$N^1$,$N^1$-Dimethyl-$N^2$-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;

6-Methyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;

$N^1$-(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-$N^2$,$N^2$-dimethyl-1,2-ethanediamine;

$N^1$-[6-(2-Methoxyethoxy)-1,4-dioxido-1,2,4-benzotriazin-3-yl]-$N^2$,$N^2$-dimethyl-1,2-ethanediamine;

$N^1$,$N^1$-Dimethyl-$N^2$-(6-ethoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
6-Ethyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
2-[(3-Ethyl-1,4-dioxido-1,2,4-benzotriazin-6-yl)oxy]-N,N-dimethylethaneamine;
3-Ethyl-6-[3-(4-morpholinyl)propoxy]-1,2,4-benzotriazine 1,4-dioxide;
6-Methyl-1,2,4-benzotriazin-3-amine 1,4-dioxide; and
their pharmacologically acceptable salts thereof.

4 A method of therapy for treating cancer including the step of administering a 1,2,4-benzotriazine-1,4-dioxide compound as claimed in claim 2 or claim 3 in a therapeutically effective amount to tumour cells in a subject.

5 The method as claimed in claim 4 wherein the tumour cells are in a hypoxic environment.

6 The method as claimed in claim 4 or claim 5 further including the step of administering radiotherapy to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 2 or claim 3 to the tumour cells.

7 The method as claimed in claim 6 further including the step of administering one or more chemotherapeutic agents to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 2 or claim 3 to the tumour cells.

8 The method as claimed in claim 7 wherein the one or more chemotherapeutic agents is selected from Cisplatin or other platinum-based derivatives, Temozolomide or other DNA methylating agents, cyclophosphamide or other DNA alkylating agents, Doxorubicin, mitoxantrone, camptothecin or other topoisomerase inhibitors, Methotrexate, gemcitabine or other antimetabolites and/or Docetaxel or other taxanes.

9 A method of radiosensitising in a subject tumour cells of solid tumours in hypoxic conditions in vivo, comprising the steps of:
(a) administering to the subject a pharmaceutical composition in an amount sufficient to produce radiosensitivity in the tumour cells, the composition comprising a 1,2,4-benzotriazine-1,4 dioxide obtained by the method defined in claim 1; and

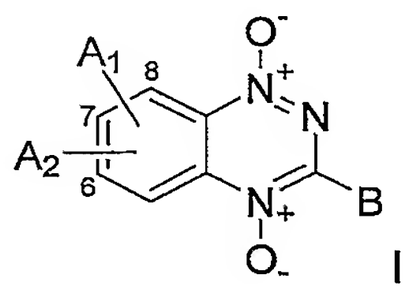
(b) subjecting the tumour cells to radiation.

10 The use in the manufacture of a medicament of a therapeutically effective amount of a1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 2 or claim 3 for the treatment of tumour cells in a subject.

11 The use as claimed in claim 10 wherein the tumour cells are in a hypoxic environment.

12 A pharmaceutical composition including a therapeutically effective amount of a 1,2,4-benzotriazine-1,4-dioxide as defined in claim 2 or claim 3 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

13 A 1,2,4-benzotriazine-1,4-dioxide compound of Formula I



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wherein

$A_1$ or $A_2$ represent independently an H or R substituent at positions 6, 7 or 8 and/or an OR substituent at positions 6 or 8

wherein each R independently represents a $C_{1-4}$ alkyl or cyclic $C_3$-$C_8$ alkyl optionally substituted with substituents selected from OH, OMe, or $NR^1R^1$ and wherein each $R^1$ is independently selected from H or a $C_{1-3}$ alkyl or the $R^1R^1$ substituents together form a morpholine ring;

B represents $NHR^2$ or $R^3$;

wherein $R^2$ is a $C_{1-3}$ alkyl optionally substituted with substituents selected from OH, OMe, or $NR^4R^4$

wherein $R^3$ is selected from a $C_{1-3}$ alkyl optionally substituted with OH, OMe,

wherein each $R^4$ is independently selected from H, a $C_{1-3}$ alkyl, optionally substituted with OMe, or $R^4R^4$ together form morpholine;

or a pharmacologically acceptable salt thereof, and;

having the characteristics

(a) a solubility greater than or about 2mM in culture medium; and

(b) an HT29 anoxic $IC_{50}$ for a 4hr exposure to the 1,2,4-benzotriazine-1,4-dioxide of less than or about 40 μM;

(c) a hypoxic cytotoxicity ratio (HCR) greater than about 20 for the HT29 cell line; and

(d) a penetration half distance (PHD) greater than or about 27 μm, and

(e) the area under the plasma concentration time curve for free 1,2,4-benzotriazine-1,4-dioxide (unbound to plasma proteins), $AUC_f$, is greater than about 2 times the HT29 anoxic $IC_{50}\times t$ where $IC_{50}\times t$ is the product of concentration × exposure time for 50% inhibition of cell proliferation

and wherein for said 1,2,4-benzotriazine-1,4-dioxide at least one of the characteristics (a) to (e) exceeds the activity of the equivalent characteristic of Tirapazamine; and

with the proviso that $A_1$ and $A_2$ do not both represent H when B represents $CH_2CH_3$ or $CH_2CH_2OCH_3$; and

with the further proviso that when $A_1$ represents H and $A_2$ represents 7-Me then B cannot represent $NH(CH_2)_2NMe_2$.

14 A 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in claim 13 selected from

$N^1$,$N^1$-Dimethyl-$N^2$-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;

6-Methyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;

$N^1$-(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-$N^2$,$N^2$-dimethyl-1,2-ethanediamine;

$N^1$-[6-(2-Methoxyethoxy)-1,4-dioxido-1,2,4-benzotriazin-3-yl]-$N^2$,$N^2$-dimethyl-1,2-ethanediamine;

$N^1$,$N^1$-Dimethyl-$N^2$-(6-ethoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;

6-Ethyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;

2-[(3-Ethyl-1,4-dioxido-1,2,4-benzotriazin-6-yl)oxy]-N,N-dimethylethaneamine;

3-Ethyl-6-[3-(4-morpholinyl)propoxy]-1,2,4-benzotriazine 1,4-dioxide;

6-Methyl-1,2,4-benzotriazin-3-amine 1,4-dioxide; and

their pharmacologically acceptable salts thereof.

15 A method of therapy for treating cancer including the step of administering a 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in claim 13 or claim 14 in a therapeutically effective amount to tumour cells in a subject.

16 The method as claimed in claim 15 wherein the tumour cells are in a hypoxic environment.

17 The method as claimed in claim 15 or claim 16 further including the step of administering radiotherapy to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound as defined in claim 13 or claim 14 to the tumour cells.

18 The method as claimed in claim 17 further including the step of administering one or more chemotherapeutic agents to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in claim 13 or claim 14 to the tumour cells.

19 The method as claimed in claim 18 wherein the one or more chemotherapeutic agents is selected from Cisplatin or other platinum-based derivatives, Temozolomide or other DNA methylating agents, cyclophosphamide or other DNA alkylating agents, Doxorubicin, mitoxantrone, camptothecin or other topoisomerase inhibitors, Methotrexate, gemcitabine or other antimetabolites and/or Docetaxel or other taxanes.

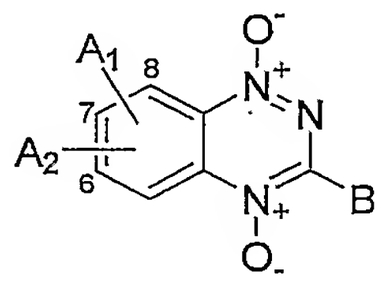
20 A method of radiosensitising in a subject tumour cells of solid tumours in hypoxic conditions in vivo, comprising the steps of:
(a) administering to the subject a pharmaceutical composition in an amount sufficient to produce radiosensitivity in the tumour cells, the composition comprising a 1,2,4-benzotriazine-1,4 dioxide as claimed in claim 13 or claim 14; and
(b) subjecting the tumour cells to radiation.

21 The use in the manufacture of a medicament of a therapeutically effective amount of a 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in any claim 13 or claim 14 for the treatment of tumour cells in a subject.

22 The use as claimed in claim 21 wherein the tumour cells are in a hypoxic environment.

23 A pharmaceutical composition including a therapeutically effective amount of a 1,2,4-benzotriazine-1,4-dioxide of Formula I as defined in claim 13 or claim 14 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

24 A compound of Formula I or a pharmacologically acceptable salt thereof,



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wherein

$A_1$ or $A_2$ represent independently an H or R substituent at positions 6, 7 or 8 and/or an OR substituent at positions 6 or 8

wherein each R independently represents a $C_{1-4}$ alkyl or cyclic $C_3$-$C_8$ alkyl optionally substituted with substituents selected from OH, OMe, or $NR^1R^1$ and wherein each $R^1$ is independently selected from H or a $C_{1-3}$ alkyl or the $R^1R^1$ substituents together form a morpholine ring;

B represents $NHR^2$ or $R^3$;

wherein $R^2$ is a $C_{1-3}$ alkyl optionally substituted with substituents selected from OH, OMe, or $NR^4R^4$

wherein $R^3$ is selected from a $C_{1-3}$ alkyl optionally substituted with OH, OMe,

wherein each $R^4$ is independently selected from H, a $C_{1-3}$ alkyl, optionally substituted with OMe, or $R^4R^4$ together form a morpholine ring;

or a pharmacologically acceptable salt thereof, and

with the proviso that $A_1$ and $A_2$ do not both represent H when B represents $CH_2CH_3$ or $CH_2CH_2OCH_3$; and

with the further proviso that when $A_1$ represents H and $A_2$ represents 7-Me then B cannot represent $NH(CH_2)_2NMe_2$.

25 A compound of Formula I as claimed in claim 24 wherein $A_1$ represents Me, Et, OMe, OEt, or $OCH_2CH_2OMe$; $A_2$ represents H and B represents Me, Et, $CH_2CH_2OH$, $CH_2CH_2OMe$, $NHCH_2CH_2NMe_2$, $NHCH_2CH_2$Nmorpholine, or $NHCH_2CH_2CH_2$Nmorpholine.

26 A compound of Formula I as defined in claim 24 or claim 25 wherein $A_1$ represents $CH_2CH_2NMe_2$, $CH_2CH_2$Nmorpholine, $CH_2CH_2CH_2$Nmorpholine,

$OCH_2CH_2NMe_2$, $OCH_2CH_2$Nmorpholine or $OCH_2CH_2CH_2$Nmorpholine and B represents Me, Et, $CH_2CH_2OH$ or $CH_2CH_2OMe$.

27 A 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in claim 24 selected from
$N^1$,$N^1$-Dimethyl-$N^2$-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
6-Methyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
$N^1$-(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-$N^2$,$N^2$-dimethyl-1,2-ethanediamine;
$N^1$-[6-(2-Methoxyethoxy)-1,4-dioxido-1,2,4-benzotriazin-3-yl]-$N^2$,$N^2$-dimethyl-1,2-ethanediamine;
$N^1$,$N^1$-Dimethyl-$N^2$-(6-ethoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine;
6-Ethyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-dioxide;
2-[(3-Ethyl-1,4-dioxido-1,2,4-benzotriazin-6-yl)oxy]-N,N-dimethylethaneamine;
3-Ethyl-6-[3-(4-morpholinyl)propoxy]-1,2,4-benzotriazine 1,4-dioxide;
6-Methyl-1,2,4-benzotriazin-3-amine 1,4-dioxide; and
their pharmacologically acceptable salts thereof.

28 A method of therapy for treating cancer including the step of administering a 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as claimed in any one of claims 24 to 27 in a therapeutically effective amount to tumour cells in a subject.

29 The method as claimed in claim 28 wherein the tumour cells are in a hypoxic environment.

30 The method as claimed in claim 28 or claim 29 further including the step of administering radiotherapy to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in any one of claims 24 to 27 to the tumour cells.

31 The method as claimed in claim 30 further including the step of administering one or more chemotherapeutic agents to the tumour cells before, during or after the administration of the 1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in any one of claims 24 to 27 to the tumour cells.

32 The method as claimed in claim 31 wherein the one or more chemotherapeutic agents is selected from Cisplatin or other platinum-based derivatives, Temozolomide or other DNA methylating agents, cyclophosphamide or other DNA alkylating agents, Doxorubicin, mitoxantrone, camptothecin or other topoisomerase inhibitors, Methotrexate, gemcitabine or other antimetabolites and/or Docetaxel or other taxanes.

33 A method of radiosensitising in a subject tumour cells of solid tumours in hypoxic conditions in vivo, comprising the steps of:
(a) administering to the subject a pharmaceutical composition in an amount sufficient to produce radiosensitivity in the tumour cells, the composition comprising a 1,2,4-benzotriazine-1,4 dioxide as claimed in any one of claims 24 to 27; and
(b) subjecting the tumour cells to radiation.

34 The use in the manufacture of a medicament of a therapeutically effective amount of a1,2,4-benzotriazine-1,4-dioxide compound of Formula I as defined in any one of claims 24 to 27 for the treatment of tumour cells in a subject.

35 The use as claimed in claim 34 wherein the tumour cells are in a hypoxic environment.

36 A pharmaceutical composition including a therapeutically effective amount of a 1,2,4-benzotriazine-1,4-dioxide of Formula I as defined in any one of claims 24 to 27 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.